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The traditional use of *Vachellia nilotica* for sexually transmitted diseases is substantiated by the antiviral activity of its bark extract against sexually transmitted viruses.

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ABSTRACT

Ethnopharmacological relevance: Vachellia (Acacia) nilotica and other plants of this genus have been used in traditional medicine of Asian and African countries to treat many disorders, including sexually transmitted diseases, but few studies were performed to validate their anti-microbial and anti-viral activity against sexually transmitted infections.

Aim of the study: The present study was undertaken to explore whether the ethnomedical use of *V.nilotica* to treat genital lesions is substantiated by its antiviral activity against the human immunodeficiency virus (HIV), the herpes simplex virus (HSV) and the human papillomavirus (HPV).

Materials and methods: The antiviral activity of *V.nilotica* was tested *in vitro* by virus-specific inhibition assays using HSV-2 strains, sensible or resistant to acyclovir, HIV-1IIIb strain and HPV-16 pseudovirion (PsV). The potential mode of action of extract against HSV-2 and HPV-16 was further investigated by virus inactivation and time-of-addition assays on cell cultures.

Results: *V.nilotica* chloroform, methanolic and water bark extracts exerted antiviral activity against HSV-2 and HPV-16 PsV infections; among these, methanolic extract showed the best EC50s with values of 4.71 and 1.80 µg/ml against HSV-2 and HPV-16, respectively, and it was also active against an acyclovir-resistant HSV-2 strain with an EC50 of 6.71 µg/ml. By contrast, no suppression of HIV infection was observed. Investigation of the mechanism of action revealed that the methanolic extract directly inactivated the infectivity of the HPV-16 particles, whereas a partial virus inactivation and interference with virus attachment (EC50 of 2.74 µg/ml) were both found to contribute to the anti-HSV-2 activity.

Conclusions: These results support the traditional use of *V.nilotica* applied externally for the treatment of genital lesions. Further work remains to be done in order to identify the bioactive components.

1. Introduction

Vachellia nilotica, widely known by the taxonomic synonym *Acacia nilotica* (L.), belongs to family *Fabaceae* of genus Acacia containing an excess of 1350 species (Seigler et al., 2003). It is an ornamental and medicinal plant, that grows to 14-17 meters in height and 2-3 meters in diameter. It is widespread, distributed throughout tropical and sub-tropical regions of Africa, Middle East and Indian subcontinent (Bargali and Bargali, 2009; Malviya et al., 2011; Ali et al., 2012). *A. nilotica* is a rich source of many secondary metabolites, mainly condensed tannins, flavonoids, alkaloids. All parts of the plant (pods, bark, leaves, roots, flower, gum, branches and seeds) have been used in traditional medicine of India, Pakistan and African countries, as Kenya, Zimbabwe and Sudan, for the treatment of enteric and respiratory ailments, children's fevers, toothache and eye complaints (Kaur et al., 2005; Ali et al., 2012; Rather et al., 2015). Interestingly, *A. nilotica* and other plants of Acacia genus have been used by traditional healers to treat sexually transmitted infections (STI) and HIV/AIDS-related diseases (Kambizi et al., 2001; Chinsembu et al., 2016). For instance, in Guruvedistric, Zimbawe, the *A. nilotica* fruits are grounded into powder and applied of penile sores a kind of lesions that can be caused either by a bacterial infection like syphilis or by a viral infection like herpes simplex virus (Kambizi et al., 2001). In Livingstone, Zambia, herbalists use *A. nilotica, A.albida, A.polyacantha, A.ataxacantha and A. Schweinfurthii* to treat STI like syphilis, gonorrhoea and other AIDS-related infections (Chinsembu et al., 2016). Recently, various extracts of *A.nilotica* have been investigated for their antibacterial (Vijayasanthiet al., 2011;Amin et al., 2013; Oladosu et al., 2013; Baiet al., 2015; Shekaret al., 2015), antiprotozoal (Jigam et al., 2010; Mann et al., 2011; Bapna et al., 2014; Alli et al., 2016), antifungal (Satish et al., 2007; Mbatchou et al., 2012; Rai et al., 2014), and antiviral activities (Hussein et al., 1999; Hussein et al., 2000; Asres et al., 2005; Rehman et al., 2011; Raheel et al., 2013; Sharma et al., 2014). The traditional use against sexually transmitted diseases and its antiviral potential prompted us to investigate on the *V. nilotica* antiviral activity against three sexually transmitted viruses, namely the human immunodeficiency virus

(HIV), the herpes simplex virus (HSV) and the human papillomavirus (HPV).

STIs caused by bacterial and protozoan pathogens, including syphilis, gonorrhoea, Chlamydia, and trichomoniasis, are generally curable. By contrast, HIV, HSV and HPV infections are currently incurable through drug treatment (http://www.who.int/en/).

According to WHO, around 36.7 million people were living with HIV at the end of 2015. The "high activity antiretroviral therapy" or HAART has transformed a terminal illness into a chronically managed disease where patients can have a near-healthy quality of life. However, to date, there is not an effective vaccine or a cure to eradicate the established infection, firstly for the high mutation rate of viral genome and its ability to escape the host immune response, secondly for the integration of provirus in cellular DNA and, thirdly for the presence of cellular and anatomical HIV reservoirs where virus maintains low level of viral replication, despite treatment (Svicher et al., 2014; Sarmati et al. 2015). Genital herpes infection, mainly caused by HSV type 2, affects more than 500 million people worldwide. These infections are mostly asymptomatic but can also cause painful blisters or ulcers in the genital or anal area (Roitzman et al., 2007). In addition, HSV-2 infection can increase the risk of HIV acquisition by approximately three-fold and genital herpes can occur in 60-90% of HIV-infected people (Freeman et al., 2006; Feng et al., 2013). HSV infections are lifelong for ability of virus to establish latency in the neurons of the sensory ganglia, therefore antiviral drugs, such as acyclovir, famciclovir, and valacyclovir, can reduce the severity and frequency of symptoms, but cannot cure the infection (Cunningham et al., 2006). Another important sexually transmitted virus is HPV, especially types 16 and 18, that cause 528000 cases of cervical cancer and 266000 deaths each year. To date, no anti-HPV drugs are available to cure HPV lesions and current treatments are ablative. If for HIV and HSV-2 infections no vaccines are currently available, in recent years two safe HPV vaccines have been introduced in routine immunization programmes in 65 countries (http://www.who.int/en/). Populations of developing countries with the highest rates of STIs often do not have access to adequate health services. Therefore, despite the presence of antiviral drugs for HIV and HSV-2 and HPV vaccines, their very high costs limit the administration

in people living in low-socioeconomic settings. In this contest, medicinal plants, as *Vachellia nilotica*, have been extensively used to treat infectious diseases in India and Africa. This study was undertaken to explore whether the ethnomedical use of *V. nilotica* to treat STI could be substantiated by an antiviral activity against HSV-2, HPV-16 and HIV-1. Here, we report on the cytotoxicity, the antiviral spectrum of activity of three *V.nilotica* bark extracts and the probable mechanisms of antiviral action of *V. nilotica* methanol bark extract.

2. Materials and methods

2.1. Plant material

The bark of *Vachellianilotica* L. were collected from the medicinal plant garden of Birla Institute of Technology, Mesra, Ranchi and authenticated by Botanical Survey of India, Central National Herbarium, Botanical Garden, Howrah (Letter No. CNH/Tech.II/2015/18/275 dated 23-04-2015). A specimen has been deposited at the herbarium (voucher no, SM-5).

2.2. Preparation of extracts

The fresh bark of *Vachellia nilotica* L. was completely dried at 40–45°C and pulverized in a knife grinder to fine powder (250 μm- particle diameters). The powdered bark (250 g) was then successively extracted by soxhlation using chloroform and methanol. Water extract of bark was prepared by decoction. Extracts were filtered and evaporated to dryness using a rotator evaporator under controlled temperature and reduced pressure. The obtained extracts were lyophilized and stored in desiccators (Silva et al., 2010).

2.3. Phytochemical investigations of V. nilotica methanolic extract

The plant extract was submitted to preliminary phytochemical screening according to the methods previously described (Manosroi et al., 2010). Briefly, Dragendorff's reagent, Ammonia solution, Killer kiliani test, Shinoda test, Molisch test, $FeCl_3$, Frothing test, and Salkowski test were performed to detect alkaloids, anthraquinone glycosides, cardiac glycosides, flavonoids, carbohydrate, tannins, saponins and steroids/terpenoids, respectively.

2.4. Cells

African green monkey kidney cells (Vero) (ATCC CCL-81) were cultured in Eagle's minimal essential medium (MEM) (Gibco/BRL, Gaithersburg, MD) supplemented with heat inactivated 10% fetal calf serum (FCS) (Gibco/BRL). The human cervical carcinoma cell lines HeLa (ATCC CCL-

2) were grown as monolayers in Dulbecco's modified Eagle's medium (DMEM) (Gibco-BRL, Gaithersburg, MD) supplemented with heat-inactivated 10% FCS. The 293TT cell line, derived from human embryonic kidney cells transformed with the simian virus 40 (SV40) large T antigen, was cultured in DMEM supplemented with heat-inactivated 10% FCS and nonessential amino acids. 293TT cells allow high levels of protein to be expressed from vectors containing the SV40 origin due to over-replication of the expression plasmid (Buck et al., 2005). C8166 CD4+T lymphoblastoid cell line was mantained in RPMI 1640 (GIBCO, Grand Island, NY) supplemented with 10% FCS at an optimal cell density of 0.5 to $1.5x10^6$ cells/ml. All media were supplemented with1% antibiotic-antimycotic solution (Zell Shield, Minerva Biolabs GmbH, Berlin, Germany) and cells were grown at 37°C in an atmosphere of 5% of CO_2.

2.5.Viruses

HSV-2 strain (ATCC VR-540) and a HSV-2 strain with phenotypic resistance to acyclovir were used. The resistant strain was generated by serial passage in the presence of increasing concentrations of acyclovir as previously described by Field et al. (1980). The resistant virus was then plaque purified, and the antiviral susceptibility was tested as described in Donalisio et al. (2016). Viral strains were propagated, collected and titrated by plaque assay on Vero cells. HIV-1IIIb strain stock was prepared in C8166 cells as previously described (Bon et al., 2013). The viral stocks were titrated with an HIV-1 gag p24 antigen ELISA kit (Biomerieux, Marcy L'Etoile, France).

2.6. *HPV PsV production*

Plasmids and 293TT cells used for pseudovirus (PsV) production were kindly provided by John Schiller (National Cancer Institute, Bethesda, MD) or bought at Addgene (Cambridge, MA). Detailed protocols and plasmid maps for this study can be seen at http://home.ccr.cancer.gov/lco/pseudovirusproduction.htm. HPV-16 PsVs were produced according to previously described methods (Buck et al., 2005). Briefly, 293TT cells were transfected with plasmids expressing the papillomavirus major and minor capsid proteins (L1 and

L2, respectively), together with a reporter plasmid expressing the green fluorescent protein (GFP), named pfwB. Capsids were allowed to mature overnight in cell lysate; the clarified supernatant was then loaded on top of a density gradient of 27 to 33 to 39% Optiprep (Sigma-Aldrich, St. Louis, MO) at room temperature for 3 h. The material was centrifuged at 28,000 rpm for 18h at room temperature in an SW41.1 rotor (Beckman Coulter, Inc., Fullerton, CA) and then collected by bottom puncture of the tubes. Fractions were inspected for purity as described in Cagno et al. (2015) and the L1 protein content of PsV stocks was determined by comparison with bovine serum albumin standards in Coomassie-stained SDS-polyacrylamide gels.

2.7. HSV inhibition assay

The effect of extracts on HSV-2 infections was evaluated by plaque reduction assay as described in Donalisio et al. (2014). Briefly, increasing concentrations of extracts were added to cells before, during and after infection. HSV-2 or acyclovir resistant HSV-2 were used at MOI of 0.0003 pfu/cell. After 24h of incubation at 37°C, cells were fixed and stained with 0.1% crystalviolet in 20% ethanol and viral plaques counted. The effective concentrations producing 50% and 90% reduction in plaque formation (EC50 and EC90) were determined using Prism software by comparing drug-treated with untreated wells. The selectivity index (SI) was calculated by dividing the CC50 by the EC50 value. As control, Vero cells were treated with Acyclovir (Sigma-Aldrich).

2.8. HPV GFP-based assay

HeLa cells were seeded in 96-well plates at a density of 8,000 cells/well in 100 µl of DMEM supplemented with 10% FBS. The next day, serial dilutions of extracts were added to preplated cells for 2 hours at 37°C, after the incubation time the extracts were removed and mixtures of extracts and dilutions of PsV stocks at a multiplicity of infection (MOI) of 0.05 (determined by calculation of the fraction of cells positive for reporter protein expression in untreated cells) were added. The percent of infection was calculated as described elsewhere (Savoia et al., 2010). As control, cells were infected and treated with heparin (Laboratori Derivati Organici S.p.A., Milan, Italy).

187 *2.9. HIV inhibition assay*

188 Viral stocks were assessed at concentration of 1000 ng p24/ml. HIV-1_{IIIb} laboratory strains (5 ng/ml
189 of HIV-1 gag p24) was pre-incubated for one hour at 37°C with increasing concentrations of
190 extracts (0, 0.025, 0,25, 2,5, 25 μg/ml) and then added to C8166 cells ($0.5x10^6$ cells/ml) for 2 hours
191 at 37°C. After three washes in PBS, the cells were seeded at $5x10^5$ cells/ml into fresh medium plus
192 the drug concentration used in the pre-incubation. The HIV-1 gag p24 amount was determined
193 seven days post-infection in the culture supernatants with the HIV-1 p24 antigen ELISA kit
194 (Biomerieux). Mock-infected C8166 cells in medium with or without DMSO were used as the
195 negative controls. As further control, we have treated the HIV-1_{IIIb}-infected C8166 with serial
196 dilutions of Tenofovir (NIBSC, London, UK).

197 *2.10. Cell viability assay*

198 HeLa and Vero cells were seeded in 96-well plates and, the next day, treated with serially diluted
199 extracts. After 24h (Vero cells) or 72 h (HeLa cells) of incubation cell viability was determined
200 using the Cell Titer 96 Proliferation Assay Kit (Promega, Madison, WI, USA), according to the
201 manufacturer's instructions. Absorbances were measured using a Microplate Reader (Model 680,
202 BIORAD) at 490 nm. The percent of viability was calculated in comparison with the untreated
203 control, where medium and extract solvent was added. The viability of the C8166 cells in presence
204 of scalar concentrations of extractor Tenofovir was analysed by the Trypan Blue exclusion
205 technique at day 7 post-infection.

206 *2.11. Mechanism of action assays against HSV-2 infection*

207 Vero cells were seeded in 96-well plates or 24-well plates in MEM supplemented with 10% FBS,
208 for virus inactivation assay and all other assays, respectively. The following day:

209 - In virus inactivation assay, extract (33μg/ml) was added to aliquots of 10^5 PFU HSV-2 and
210 incubated at 37°C for 2 h. After incubation, samples were titrated on Vero cells at high
211 dilutions, at which the extract was not active.

- In time of addition assay, serial dilutions of extract were added on cells 2h before infection or during infection or post-infection. After the incubation time described before, viral plaques were counted.
- In attachment assay serial dilutions of extract or heparin were mixed with HSV-2 and added to cooled cells and incubated for 2h at 4°C to ensure viral attachment but not entry. After two gentle washes, cells were overlaid with 1.2% methylcellulose medium, shifted to 37°C for 24h and successively plaques were counted.
- In entry assay, HSV-2 at MOI of 0.01 PFU/cell was adsorbed for 2h at 4°C on pre-chilled confluent cells. Cells were then washed with cold MEM three times to remove unbound virus, treated with different concentrations of extract, and incubated for 3h at 37°C. Un-penetrated viruses were inactivated with acidic glycine for 2 min at room temperature. Cells were then washed with warm medium three times and treated.

2.12. HPV inactivation assay

HeLa cells were seeded in 96-well plates in 100 µl of DMEM supplemented with 10% FBS. The following day a mixture of 11 µg/ml of extract and HPV-16 PsV or HPV-16 PsV (10^5 focus forming units) alone was incubated for 2 hours at 37°C and subsequently titrated on cells. 72 hours later the infection was measured.

2.13. Data analysis

All results are presented as the mean values from three independent experiments. The EC50 and EC90 values for inhibition curves were calculated by regression analysis using the software Graph-Pad Prism version 5.0 (GraphPad Software, SanDiego, California, U.S.A.) by fitting avariable slope-sigmoidal dose–response curve. For virus inactivation assays the viral infectivity in presence and absence of extract was compared using a one-way analysis of variance (ANOVA) followed by

235 Bonferroni test, if P values showed significant differences in virus titers. Significance was set at the
236 95% level.

237

Results and discussion

V.nilotica chloroform, methanol and water extracts were prepared obtaining w/w extraction yields of 6.27, 9.37 and 10.25% w/w, respectively, as previously described. In a first set of experiments, we tested the antiviral activity of three *V.nilotica* bark extracts against HSV-2, HPV-16 and HIV-1. Since extracts were resuspended in DMSO to a final concentration of 25 mg/ml before use, control samples with equal volumes of DMSO were included in the assay in order to rule out any cytotoxic effect ascribable to the solvent.

Table 1. Antiviral activity of *V.nilotica* bark extracts against HSV-2, HPV-16, HIV-1and acyclovir resistant HSV-2 strain

Virus	Extract	EC50*(µg/ml) – 95% C.I. #	EC90§(µg/ml)– 95% C.I.	CC50†(µg/ml)	SI‡
HSV-2	Chloroform	12.3 (7.72-19.6)	35.9 (11.4 -62.7)	189	15.4
	Methanol	4.71 (3.11-7.12)	8.07 (2.66-24.4)	144	30.6
	Water	10.2 (7.95-12.9)	20.1 (8.77-46.0)	190	18.6
	Acyclovir	0.64 (0.48-0.86)	3.17 (1.69-5.95)	>300	>468
HPV-16	Chloroform	3.89 (1.69-8.88)	23.5 (4.15-43.4)	211	54.2
	Methanol	1.80 (1.42-2.27)	5.48 (3.63-8.28)	58.7	32.6
	Water	5.51 (4.58-6.63)	15.1(10.1-22.1)	115	20.9
	Heparin	2.20 (1.83-3.01)	4.3 (3.05-5.01)	>300	>136
HIV-1$_{IIIb}$	Chloroform	-	-	74	-
	Methanol	-	-	74	-
	Water	-	-	74	-
	Tenofovir	0.21 (0.12 -0.37)	1.09(0.36- 3.29)	>14.3	>68.3
HSV-2 ACVr¥	Methanol	6.71 (3.85-11.7)	10.5 (5.99-18.5)	144	21.5
	Acyclovir	62.0 (57.7-65.2)	> 500	> 300	> 5.00

EC50* half maximal effective concentration

C.I.# confidence interval

250 EC90[§]90%effective concentration
251 CC50[†] half maximal cytotoxic concentration
252 SI[‡] selectivity index
253 [¥] HSV-2 acyclovir resistant strain
254
255
256 Results shown in Table 1 reveal that the three extracts were active against HSV-2 and HPV-16 PsV
257 although at different extents. Chloroform, methanolic and water extracts inhibited HSV-2 infection
258 with selectivity indexes of 15.4, 30.6 and 18.6, respectively, whereas they exerted anti-HPV-16
259 activity with selectivity indexes of 54.2, 32.6 and 20.9, respectively. These data indicated that the
260 antiviral activity was not due to cytotoxicity. Acyclovir and heparin were tested in parallel as
261 reference inhibitors for HSV-2 and HPV-16 respectively and exerted the expected antiviral activity.
262 By contrast, when *V. nilotica* bark extracts were tested on C8166 T CD4+ lymphoblastoid cells
263 against HIV-1_{IIIb} strain infection, no suppression of HIV infection was observed. In the same assay,
264 the reference drug tenofovir strongly inhibited HIV-1 infection in a dose-response manner (Table
265 1). The lack of anti-HIV-1 activity of extracts is in accord with the study by Hussein et al., that
266 reported no HIV-1inhibitory activity of methanol and water bark extracts from Sudanese *Acacia*
267 *nilotica*. However, it must be noted that, despite the lack of HIV-1 inhibitory activity, the extracts of
268 the bark and the pods have been reported to exert an anti-HIV-1 protease activity (Hussein et al.,
269 1999).
270 Although aqueous extract is mainly used in traditional medicine to treat STIs, our results
271 demonstrated that methanolic extract is the most active one with the lowest EC50 of 4.71 and 1.80
272 µg/ml against HSV-2 and HPV-16 infections, respectively. Therefore, the *V. nilotica* bark
273 methanolic extract was chosen for further studies aimed at investigating its major mechanism(s) of
274 action.
275 Of note, the methanolic extract proved active also against an acyclovir-resistant HSV-2 strain
276 (HSV-2 ACV-r) that was previously generated in our laboratory (Donalisio et al., 2016). This

finding might indicate that the active components of the extract act through a different mechanism of action to that of acyclovir, an inhibitor of viral DNA polymerase.

To explore whether the extract directly inactivates HSV-2 virus particles, 10^5 PFU of HSV-2 and 33 µg/ml of extract (a dose inhibiting 90% of infectivity in previous assays, i.e. EC_{90}) were incubated at 37°C for 2 h and, then, the samples were titrated on cells at high dilutions, at which the extract was no longer active. A 66% of inhibition of viral titers was observed indicating that the anti-HSV-2 activity of the extract depends, at least in part on the inactivation extracellular virus particles (data not shown).

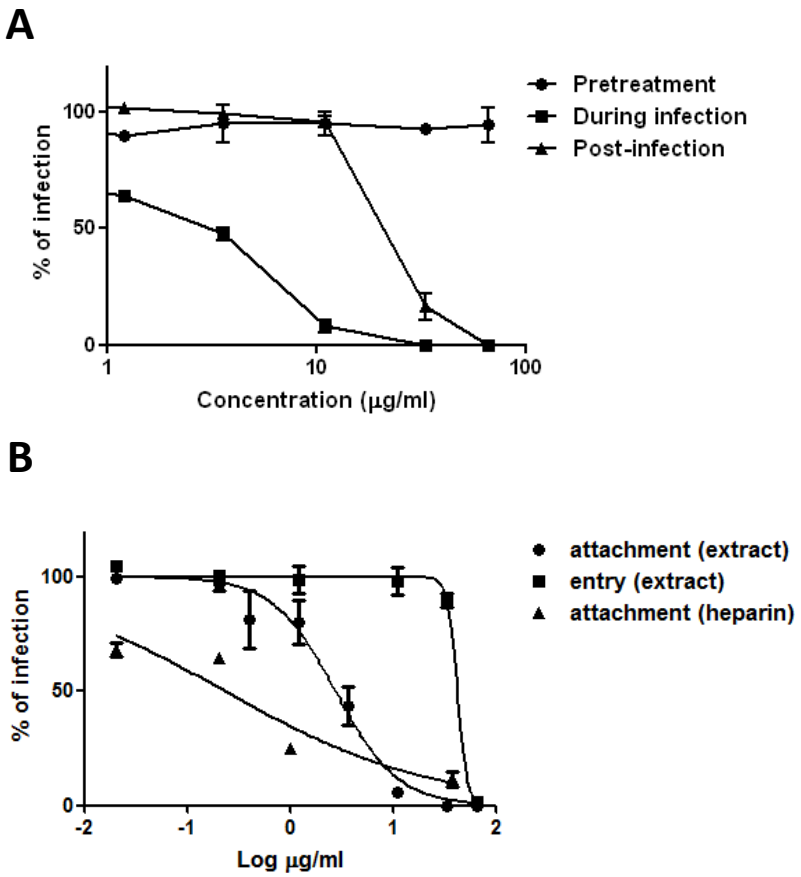

Fig. 1. Mode of anti-HSV-2 activity of methanol bark extract. A) Time-of-addition assays. Cells were treated with methanol bark extract for 2 h before infection (pre-treatment), for 2 h during infection or by adding the extract immediately after infection (post-infection). The number of plaques in the treated samples is expressed as a percentage of control (DMSO treated). B) HSV-2 entry and attachment assays. The methanol bark extract of *V. nilotica* was added to the cell culture during virus-cell binding (attachment assay) or virus-cell penetration (entry assay). Heparin was used as a known inhibitor of attachment. The number of viral plaques in the treated samples is

294 expressed as a percentage of control (DMSO treated). Each point represents mean and SEM for
295 triplicates.

296

297 To investigate if bark extract is also able to target cell-surface-virus interactions or intracellular
298 steps of the HSV-2 replicative cycle, we performed a set of time-of-addition assays in which the
299 sample was added to the cells only before (pre-treatment), during, or after infection, and viral
300 plaques were counted after an incubation time of 24 h. Figure 1A shows that pre-treatment with the
301 bark extract did not affect cell susceptibility to HSV-2 infection thus excluding the possibility that
302 components of the extract form stable interactions with one or more cellular components,
303 preventing their interaction with viral glycoproteins. By contrast, a strong antiviral activity was
304 observed when the extract was added during infection with EC_{50} values of 1.94μg/ml. A modest
305 dose-dependent effect occurred when the extract was added post-infection (EC_{50} of 22.5μg/ml).
306 These data bring out several considerations: 1) bark extract does not target cell-surface; 2) the
307 extract inhibits probably also early steps of the virus replicative cycle since a significant reduction
308 in the number of viral plaques was also observed when the extract was added to methylcellulose
309 medium during infection. To confirm this hypothesis, an attachment and entry assays were
310 performed (Ghosh et al., 2016). As described in Figure 1B, bark extract inhibited HSV-2 attachment
311 to Vero cells generating a dose-response curve with EC50 of 2.74 μg/ml. A dose response curve
312 was also obtained treating cells with heparin, a known inhibitor of attachment (EC50: 0.23 μg/ml).
313 By contrast, performing the entry assay, a significant reduction of infectivity was only observed at
314 highest tested concentration (66 μg/ml). Then, we carried out studies to elucidate whether the
315 mechanism of action was similar against HSV-2 and HPV-16 PsV infections.
316 Interestingly, 11 μg/ml of *V. nilotica* extract was able to completely abrogate the infectivity of
317 HPV-16 pseudovirions in the virus inactivation assay indicating that bark extract exerts a virucidal
318 activity as main mechanism of action against HPV-16 (data not shown).

Summing described results, we demonstrated the ability of *V. nilotica* methanol bark extract to exert a specific virucidal action against HPV-16, whereas more than one mechanism of action against HSV-2 infection, including a partial inactivation of the infectivity of virus particles and an inhibition of viral attachment to cells. These multiple antiviral actions can be attributed to several bioactive metabolites of the plant. A preliminary phytochemical screening detected saponins and flavonoids as main constituents and tannins in traces.

Further work remains to be done to investigate which component is responsible for biological activity and elucidate their mechanisms of action.

Overall, our findings support the traditional use of *V. Nilotica* extracts for the treatment of sexually transmitted diseases due to HSV-2 and HPV-16 infections, but not due to HIV-1 infection. Interestingly, previous studies proved an antiviral activity of extracts of *Acacia nilotica* also against Hepatitis C virus, that can also be transmitted sexually, even if this mode of transmission is much less common (Hussein et al., 2000; Rehman et al., 2011; http://www.who.int/en/). Further studies are required to investigate the therapeutic potential of *V. nilotica* extracts for the treatment of HSV-2 and HPV infections.

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Conflict of interest

There is no conflict of interest associated with the authors of this paper.

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Author contributions

M.D. was responsible for all activities involving HSV-2 under the supervision of D.L.

V.C. was responsible for all activities involving HPV under the supervision of D.L.

A.C. and M.R. produced the viral stocks and were responsible of the cell viability assays.

D.G. and G.M. were responsible for all activities involving HIV.

M.G. collected and identified the plant and produced the plant extracts.

D.L. and M.G. conceived the study. M.D., V.C., and D.L. developed the interpretation of the experiments. M.G., V.C., M.G. and D.L. wrote the paper.